Modelling the transmission dynamics of cystic echinococcosis in donkeys of different ages from Tunisia

Lahmar, Samia ; Boufana, Belgees ; Jebabli, Leila ; Craig, Phillip S ; Ayari, Hayet ; Basti, Talha ; Dhibi, Mokhtar ; Torgerson, Paul R

DOI: https://doi.org/10.1016/j.vetpar.2014.06.007

Title of the manuscript: Modelling the transmission dynamics of cystic echinococcosis in donkeys of different ages from Tunisia

Author names: Samia Lahmar[a], Belgees Boufana[b], Leila Jebabli[c], Phillip S. Craig[b], Hayet Ayari[a], Talha Basti[a], Mokhtar Dhibi[a], Paul R. Torgerson[d]

Affiliations:

[a] Parasitology Laboratory, National Medicine Veterinary School, 2020 Sidi Thabet, Tunisia.

[b] Ecosystems and Environment Research Centre, School of Environment and Life Sciences, University of Salford, M5 4WT, U.K. E-mail: B.Boufana@salford.ac.uk ; P.S.Craig@salford.ac.uk

[c] Tunis Abattoir, Health Hygiene and Environment Service, Ministry of Interieur, Tunisia. leila.jebabli@hotmail.fr.

[d] Section of Epidemiology,Vetsuisse Faculty, Winterthurestrasse 270, 8057 Zurich, Switzerland. paul.torgerson@access.uzh.ch

***Corresponding author:** Samia Lahmar.
Parasitology Laboratory, National Medicine Veterinary School, 2020 Sidi Thabet, Tunisia. Tel.: +216 98383038; Fax: +216 70735562. E-mail: drlsamia@yahoo.fr

ABSTRACT

During the period from March 2006 to July 2009 a total of 2,040 slaughtered donkeys were examined for cystic echinococcosis (CE). CE prevalence in donkeys was 8.48% and the infection pressure (0.0088 infections per year) and infection rate (0.0448 cysts per year) appeared to be lower than those previously reported for cattle, sheep, dromedaries and goats in Tunisia. However, the number of cysts per infection was relatively high (5.07 cysts per infection). Among the 901 collected hydatid cysts the majority were located in the liver (89.9%), 10.09% in the lungs and 4.77% were fertile (43/901). The amplification of a fragment within the mitochondrial gene coding for cytochrome *c* oxidase subunit 1 (*cox1*) revealed that donkeys were infected with both *Echinococcus equinus* (horse strain, G4 genotype) and *E. granulosus* (sheep strain, G1 genotype). *Echinococcus granulosus* G1 developed into fertile cysts (15,112 protoscoleces/ml) with a protoscoleces viability of 65.78%. This investigation is the first detailed epidemiological report on cystic echinococcosis infection in donkeys for any endemic region.

Keywords: Echinococcosis; Donkeys; Mathematical modelling; Epidemiology; Tunisia

1. Introduction

In Tunisia cystic echinococcosis (CE) due to *Echinococcus granulosus* is a major public health problem with an incidence of 12.7 human cases/100,000 inhabitants (Chahed et al., 2010) and economic losses due to this disease are estimated at US$ 19 million/year (Majorowski et al., 2005). Domestic livestock act as intermediate hosts and are thus major reservoirs for the disease. The transmission of the parasite is carried out between canid definitive and ungulate intermediate hosts. Humans are infected through the ingestion of *Echinococcus* eggs released in faeces of carnivores and constitute a dead end host. Several reports from Tunisia on the epidemiology and genetic characterization of *E. granulosus* (*sensu lato*) in livestock such as cattle, sheep, goats and dromedaries as well as humans have been published (Farjallah et al., 2010; Dakkak, 2010; Grosso et al., 2012; Lahmar et al., 2013; Cardona and Carmena, 2013; Boufana et al., 2014). Donkeys in Tunisia constitute an estimated 65.5% (123,000) of the total national equid population (187,800) (Anon, 2006). Despite these figures there are no reports on the epidemiology of equine CE in Tunisia. It is generally assumed that potential *Echinococcus* infection of donkeys would primarily be due to *E. equinus* (horse strain, G4 genotype) which would therefore indicate a minimal public health concern as this species is believed to be non-zoonotic (Thompson and McManus,

2001). However, a recent study using molecular techniques has identified for the first time both *E. equinus* and *E. granulosus* G1 genotype from fertile hydatid cysts in Tunisian donkeys (Boufana et al., 2014). In view of these findings we investigated cystic echinococcosis in donkeys to determine the transmission dynamics of infection within different age groups. In addition, the fertility of hydatid cysts and viability of protoscoleces infecting donkeys were examined.

2. Materials and Methods

2.1. Animals and study area

The municipal abattoir of Tunis was visited daily during the periods from March 2006 to September 2007 and from July 2009 to December 2009 to examine the livers and lungs of donkeys slaughtered for animal and human consumption for the presence of hydatid cysts. A total of 2,040 donkeys from the northwest (Kef), centre-west (Siliana) and southwest (Gafsa) regions of Tunisia were included in this study. However, data on the precise origin of each animal for the three aforementioned areas was not available. Animals ranged from 2 to 25 years of age. The age of each donkey was estimated by examination of its dentition (Hadrill, 2002). Gender was also recorded for each animal.

2.2. Parasitological examination

Livers and lungs of all donkeys were examined visually and by palpation and then were cut into thin strips (about 1 cm thick) to detect and enumerate all hydatid cysts. Cyst location, external diameter and type were also recorded. Cysts were classified as viable, fertile, caseous or calcified. Total hydatid fluid was aspirated from each viable cyst and sedimented in a test tube for 15 minutes. Each cyst was incised with a scalpel blade and the laminated membrane including the germinal layer was carefully washed to recover any remaining protoscoleces, which were added to the cyst fluid sediment. For small cysts all the fully developed protoscoleces were counted under the microscope. For large cysts a proportion of the total protoscoleces and homogenised sediment was examined (100µl). The number of protoscoleces/ml was estimated by extrapolation to the total sediment volume. The mean fertility rate corresponded to the total number of the extrapolated protoscoleces/ml in each cyst divided by the total number of fertile cysts. Viability of protoscoleces was assessed for each fertile cyst using a drop of the sediment cyst fluid containing protoscoleces. A drop of 0.2% aqueous eosin solution was added to an equal volume of protoscoleces. A total of 100

protoscoleces in the sample was then scored as stained (indicating that the protoscoleces were dead) or unstained (indicating that the protoscoleces were viable) (Smyth and Barrett, 1980). The mean percentage of protoscoleces viability was calculated by dividing the total number of viable protoscoleces of each cyst by the total examined protoscoleces in all fertile cysts multiplied by100.

2.3. Molecular analysis of hydatid cyst material

Of the 43 fertile hydatid cysts, 35 hydatids belonging to 10 donkeys (22 hepatic and 13 pulmonary) were used for genotypic molecular analysis. DNA was extracted from protoscoleces or germinal layer using the Qiagen DNeasy Blood and Tissue kit according to the manufacturer's instructions. A fragment within the mitochondrial gene coding for cytochrome *c* oxidase subunit 1 (*cox 1*) (828bp) was amplified using published protocols (Nakao et al., 2000) and PCR products were commercially sequenced (Beckman Coulter Genomics, Essex, UK) in both directions. Nucleotide sequences were individually examined using FinchTV viewer (Geospiza, Seattle, WA), trimmed in Proseq 3.5 (Filatov, 2002) and blasted against the NCBI database (http://www.ncbi.nlm.nih.gov/BLAST/) (Boufana et al., 2014).

2.4. Transmission dynamics

The variation in the prevalence of hydatid cysts with age q(t), was modelled according to the model proposed by Roberts et al. (1986)

$$q(t) = 1 - \exp(-\beta t) \quad (1)$$

where β is the prevailing infection pressure in terms of number of infections per unit time (t). This equation was fitted to the data using maximum likelihood with confidence intervals estimated from the likelihood profile. The variation in the mean number of hydatid cysts in hosts with age, m(t), was analysed using the age intensity equation of Roberts et al. (1986)

$$m(t) = ht + c \quad (2)$$

m(t) is expressed in terms of infection pressure h (cysts per unit time). This was also fitted using maximum likelihood assuming a negative binomial distribution of cysts in the hosts (Torgerson et al., 2003) with confidence intervals estimated from the likelihood profile. In addition, it has been suggested that the constant of aggregation can vary with the age (or prevalence of cysts) at least in sheep (Lahmar et al., 1999). Therefore the negative binomial constant of aggregation was also modelled as varying with age according to the equations:

$$k(t) = at + b \quad (3)$$

The data were also analysed using the full non-linear model to examine the possibility of parasite induced host immunity (for equations see Roberts et al., 1986; Torgerson et al., 1998). The most parsimonious model in terms of AIC was chosen as the best model to describe the data. Differences in CE prevalence between male and female donkeys as well as cyst prevalence per infected organ (liver and lungs) were determined using the chi-square test. A *P*-value of < 0.05 was considered indicative of a statistically significant result (Schwartz, 1993). All analyses were undertaken in R (R Development Core Team, 2010). Maximum likelihood estimations and model selection utilized the bbmle (tools for general maximum likelihood estimation) package.

3. Results

3.1. Prevalence and intensity of infection

Hydatid cysts were found in 173 animals out of the 2,040 donkeys examined (8.48%). Of these 1,389 were male of which 110 were infected (7.91%), while 63 of 651 females were infected (9.67%) ($\chi 2 = 1.77$; $p = 0.183$). Prevalence (q) increased with age in years and ranged from 2.22%, 6.55%, 11.3% and 16.29% in ≤ 2, 3-9, 10-19 and ≥ 20 year age groups respectively (Fig. 1) ($\chi 2 = 73.48$; $p = 0.000000$). The parameter β (+/- 95% confidence intervals) from equation (1) was estimated at 0.0088 (0076-0.0102) infections per year. The mean number of hydatid cysts per infected donkey was 3.28, 4.15, 4.56 and 6.48 respectively for ≤ 2, 3-9, 10-19 and 20-25 year age-groups. This resulted in a mean abundance of 0.073, 0.272, 0.515 and 1.057 cysts per donkey for these respective age groups (Fig. 2).

The linear model had a lower AIC than the non-linear model and hence was a better description of the data. The linear model (2) had a non significant intercept and therefore was of the form: $m(t) = ht$. The MLE estimate of h (+/- 95% CIs) was 0.0448 (0.0353-0.0577) cysts per year. This together with the estimate for β suggested that each exposure to *Echinococcus* eggs resulted in the acquisition of a mean of 5.07 cysts. There was a significant variation of the negative binomial constant of aggregation with age. This followed equation (3), with the parameters a and b (+/- 95% CIs) estimated as 0.0021 (0.0010-0.0031) and 0.0134 (0.0042-0.0277) respectively. This is consistent with the degree of aggregation decreasing with age.

3.2. Genotyping

Molecular analysis of 35 *Echinococcus* cysts confirmed that donkeys were infected with *E. granulosus* (G1 sheep strain) or *E. equinus* (G4, horse strain) (Boufana et al., 2014). *Echinococcus equinus* and *E. granulosus* G1 genotype was identified from 22 (62.8%) and 13 (37.14%) donkey isolates respectively (Table 1). Both species were found to affect the liver and lungs and each donkey was infected with only one genotype of *Echinococcus* and no mixed infections for the same donkey were recorded.

3.3. Distribution, location, size, type and status of cysts

Of the 173 infected donkeys, hepatic cysts were seen in 141 animals, pulmonary cysts in 19 and both pulmonary and hepatic cysts in 13. Of the total 901 cysts found in infected donkeys, 810 were located in the liver (89.9%) and 91 in the lungs (10.09%) (Table 2) ($\chi 2 = 96.50$; $p = 0.000000$). Most cysts (52.71%) had a diameter between 5 and 10 mm, with 4.32% of the cysts reaching 50 mm. The miliary or early metacestode form (2-4 mm diameter) corresponded to 16.87% of cysts. Other cyst sizes varied from 12-17 mm or from 20-40 mm in diameter representing 16.87% and 9.21% of cysts respectively. All the cysts were unilocular with a thick laminated membrane and a thin germinal layer. The percentage of calcified cysts was highest (91.30%) in young donkeys (≤ 2 years) which developed a small proportion of viable cysts. For the other age-groups, the percentage of viable cysts varied between 34.58% and 41.32% but was lower than the percentage of calcified cysts reaching 59.39%, 56.61% and 65.41% respectively for the same age-groups (Table 3). Among the 35 *Echinococcus* isolates used for molecular identification, the majority of *E. granulosus* G1 hydatid cysts (12/13) were located in the lungs while most of *E. equinus* cysts (21/22) were found in the liver. *Echinococcus equinus* was identified in both young donkeys (≤ 2 to 9 year old) and in the oldest ones (≥ 20 years); whereas *E. granulosus* G1 was found in animals aged between 10 and 19 year old (Table 1).

3.4. Fertility and viability of cysts

The proportion of the total fertile cysts was low (4.77%) with a mean number of 13,190 protoscoleces/ml and a high mean percentage of protoscoleces viability (79.04%). The highest fertility rate (18,600 mean number of protoscoleces/ml) with a percentage of fertile cysts of 6.61% corresponded to the 10-19 year old age-group. In the oldest donkeys the percentage of fertile cysts (2.70%) and the fertility rate (7,850 protoscoleces/ml) was lower than those for the other age-groups. Viability of protoscoleces was similar in all age-groups (Table 3). In the lungs, the percentage of fertile cysts was higher (15.38 %) than that in the

liver (3.58%) whereas the mean number of protoscoleces/ml was more important for the fertile liver cysts (10,229) than those in the lungs (2,961) (Table 2).

Mean fertility rates of the 35 molecularly identified cysts were estimated to be 7,281, 8,812, 15,112 and 7,246 protoscoleces/ml for the age-groups ≤ 2 years (1 cyst *E. equinus*), 3 to 9 years (9 cysts *E. equinus*), 10 to 19 years (13 cysts *E. granulosus* G1) and ≥ 20 years (12 cysts *E. equinus*) respectively. Thus the mean fertility was similar for *E. equinus* and *E. granulosus* G1 isolates as was protoscoleces viability (Table 1).

4. Discussion

This is the first detailed epidemiological report on cystic echinococcosis infection in donkeys for any endemic region. This study of 2,040 donkeys showed a relatively low CE infection prevalence (8.5%, CIs 7.31-9.77) in Tunisia compared to that in equines from other countries in the region. CE prevalence was found to be 17.2% and 16.9% in donkeys from Jordan (Abo-Shehada, 1988; Mukbel et al., 2000), 10.62% in donkeys from Egypt (Haridy et al., 2008) and 17.8% in equines from Morocco (Azlaf and Dakkak, 2006). In contrast, Oge et al. (2004) reported a prevalence of just 5.7% in donkeys from Turkey and only 0.26% of donkeys from Italy were infected (Varcasia et al., 2008). The absence of a significant difference in the prevalence of CE infection between male and female donkeys seen in this study was also reported from northern Jordan (Mukbel et al. (2000). However, female donkeys from central Jordan (Abo-Shehada, 1988) and Turkey (Oge et al., 2004) harboured more cysts than males.

CE infection in this study was observed in all age-groups from the youngest donkeys (≤ 2 years; 2.2%) to the oldest age (25 years; 16.29%). In Morocco all slaughtered equines >5 years of age had a CE prevalence of 17.8% (Azlaf and Dakkak, 2006) while in Egypt donkeys over 5 years of age showed a 6.89% prevalence (Aboelhadid et al., 2013). In Jordan no donkeys of 3 years of age or less were infected (Abo-Shehada, 1988; Mukbel et al., 2000). In contrast, in eastern Turkey the majority (50%) of infected donkeys were <2 years old (Balkaya and Simsek, 2011).

CE prevalence in Tunisian donkeys increased with age and was consistent with the age prevalence model for *E. granulosus* described by Roberts et al. (1986). The increase of prevalence with age has previously been reported in cattle, sheep, goats and dromedaries

from Tunisia indicating the absence of regulating immunity (Lahmar et al., 2013). A similar study carried out utilizing the same model in donkeys from Jordan (Mukbel et al., 2000) referred to the endemic steady state (Gemmell, 1990) which implies minimal regulation of the parasite population by the intermediate host immunity.

The CE infection rate in Tunisian donkeys (0.0448 cysts per year) and the infection pressure (0.0088 infections per year) appeared to be lower than that for donkeys from Jordan which had an infection pressure of 0.48 cysts per year from 0.054 infections per year and hence 8.9 cysts per infection (Mukbel et al., 2000) whereas in Tunisian donkeys each exposure to infection resulted in a mean of 5.07 cysts. The infection pressure in donkeys in this study was also much lower than that calculated for other intermediate hosts from Tunisia such as cattle (1.06 per year; 0.141 infections per year), sheep (0.881 per year; 0.105 infections per year), goats (0.32 per year; 0.011 infections per year) and dromedaries (0.038 per year; 0.022 infections per year) (Lahmar et al., 2013). However, despite the lower number of cysts in donkeys compared to other livestock, the number of cysts per infection was relatively high (5.07 cysts per infection). This is comparable to cattle (7.51 per infection), sheep (8.39 per infection) and goats (29.09 per infection) (Lahmar et al., 2013). This suggested that donkeys were encountering the infection less frequently than other hosts, but when they did, they ingested a similar number of infectious eggs.

This work revealed that infected donkeys (82.1%) in Tunisia developed hydatid cysts in the liver only and a much lower percentage (7.5%) harboured hydatid cysts in both liver and lungs while 10.1% of CE cases occurred only in the lungs. Similar results were observed in donkeys from Egypt (Aboelhadid et al., 2013) and Jordan (Abo-Shehada, 1988), horses from Italy (Varcasia et al., 2008) and equines from Morocco (Azlaf and Dakkak, 2006). In other studies CE infection of donkeys affected exclusively the liver (Haridy et al., 2008) or the lungs (Blutke et al., 2010).

In the current survey most (63.48 %) of the hydatid cysts in donkeys were calcified particularly in young donkeys. In equines the dominance of calcified hydatid cysts was also reported from Morocco (Azlaf and Dakkak, 2006). In donkeys from Jordan Abo-Shehada (1988) observed 67.7% were non-fertile immature miliary cysts, and 10.8% were necrotic and calcified; the same author reported that 21.5% of fertile cysts were found in 47.6% of

infected donkeys harbouring viable cysts. The present study showed that 4.77% of the hydatid cysts in Tunisian donkeys were fertile (3.58% in the liver; 15.38% in the lungs).

Molecular analysis of hydatid cysts from Tunisian donkeys revealed that they were hosts to *E. equinus* and *E. granulosus* G1. Horses from Italy were also shown to harbour cysts of both *E. equinus* and *E. granulosus* (G1) although the latter were described as being infertile (Varcasia et al., 2008). In addition, infertile *E. granulosus* G1 cysts were recently described from a horse in Turkey (Utuk and Simsek, 2012). However, other studies on echinococcosis in equines have reported the presence of only *E. equinus*. *Echinococcus equinus* was molecularly confirmed in horses from Spain (Daniel Mwambete et al., 2004) and southern Germany (Blutke et al., 2010), in a captive zebra (*Equus burchellii*) from the UK (Boufana et al., 2012) and in donkeys from Egypt (Aboelhadid et al., 2013). *Echinococcus granulosus* (G1) has been shown to be the predominant strain in Tunisia occurring in sheep, cattle, goats, dromedaries, wild boars, dogs, jackals and humans (Lahmar et al., 2004; M'rad et al., 2005; Farjallah et al., 2007; Lahmar et al., 2009; Boufana et al., 2014). The present investigation revealed for the first time that *E. granulosus* (G1) can also develop into fertile cysts in donkeys with a high viability of protoscoleces (65.78%) and a mean fertility reaching 15,112 protoscoleces/ml of cyst fluid.

In conclusion, equine CE is endemic in Tunisia and is due to both *E. granulosus* (G1) and *E. equinus*. Both species were identified from fertile liver and lung hydatid cysts in animals between 2 and 20 years of age. These epidemiological findings have shown that *E. granulosus* (G1) sheep strain may have adapted to donkeys in Tunisia. Donkeys could therefore constitute a possible additional reservoir of infection in the transmission cycle of *E. granulosus* in Tunisia.

Acknowledgements

We thank Professor Mohamed Gharbi from the National School of Veterinary Medicine of Sidi Thabet for help with statistics.

Conflict of Interest

None of the authors of this paper has a conflict of interest.

306 **References**

307 Aboelhadid, S.M., El-Dakhly, K.M., Yanai, T., Fukushi, H., Hassanin, K.M., 2013. Molecular
308 characterization of *Echinococcus granulosus* in Egyptian donkeys. Vet. Parasitol. 193,
309 1-3, 292-296.
310
311 Abo-Shehada, M.N., 1988. Prevalence of hydatidosis in donkeys from central Jordan. Vet.
312 Parasitol. 30, 125-130.
313
314 Anon., 2006. Enquête sur les Structures des Exploitations Agricoles 2004/2005. Ministère de
315 l'Agriculture et des Ressources Hydrauliques. Direction Générale des Etudes et du
316 Développement Agricole, Tunisie.
317
318 Azlaf, R., Dakkak, A., 2006. Epidemiological study of the cystic echinococcosis in Morocco.
319 Vet. Parasitol. 137, 83-93.
320
321 Balkaya, I., Simsek, S., 2011. A serological survey of cystic echinococcosis in equids in East
322 of Turkey. Iranian. J. Parasitol. 6, 4, 46-50.
323
324 Blutke, A., Hamel, D., Hüttner, M., Gehlen, H., Romig, T., Pfister, K., Hermanns, W., 2010.
325 Cystic echinococcosis due to *Echinococcus equinus* in a horse from southern
326 Germany. J. Vet. Diagn. Invest. 22, 3, 458-462.
327
328 Boufana, B., Lahmar, S., Rebaï, W., Ben Safta, Z., Jebabli, L., Ammar, A., Kachti, M.,
329 Aouadi, S., Craig P.S., 2014. Genetic variability and haplotypes of *Echinococcus*
330 isolates from Tunisia. Accepted by TRSTMH.
331
332 Boufana, B., Stidworthy, M.F., Bell, S., Chantrey, J., Masters, N., Unwin, S., Wood, R.,
333 Lawrence, R.P., Potter, A., Mcgarry, J., Redrobe, S., Killick, R., Foster, A.P., Mitchell,
334 S., Greenwood, A.G., Sako, Y., Nakao, M., Ito, A., Wyatt, K., Lord, B., Craig, P.S.,
335 2012. *Echinococcus* and *Taenia* spp. from captive mammals in the United Kingdom.
336 Vet. Parasitol. 190, 95-103.
337

Cardona, G.A., Carmena, D., 2013. A review of the global prevalence, molecular epidemiology and economics of cystic echinococcosis in production animals. Vet. Parasitol. 192, 10-32.

Chahed, M.K., Bellali, H., Touinsi, H., Chérif, R., Ben Safta, Z., Essoussi, M., T. Kilani, 2010. L'incidence chirurgicale du kyste hydatique en Tunisie: Résultats de l'enquête 2001–2005 et tendance évolutive entre 1977–2005. Arch. Inst. Pasteur Tunis 87, 1–2.

Dakkak, A., 2010. Echinococcosis/hydatidosis: A severe threat in Mediterranean countries. Vet. Parasitol. 174, Issues 1–2, 24, 2–11.

Daniel Mwambete, K.D., Ponce-Gordo, F., Cuesta-bandera, C., 2004. Genetic identification and host rang of the Spanish strains of *Echinococcus granulosus*. Acta Trop. 91, 87-93.

Farjallah, S., Busi, M., Mahjoub, M.O., Slimane, B.B., Said, K., D'Amelio, S., 2007. Molecular characterization of *Echinococcus granulosus* in Tunisia and Mauritania by mitochondrial rrnS gene sequencing. Parassitologia 49, 4, 239-246.

Filatov, D.A., 2002. ProSeq: A software for preparation and evolutionary analysis of DNA sequence data sets. Mol. Ecol. Notes 2, 621-624.

Gemmell, M. A., 1990. Australasian contributions to an understanding of the epidemiology and control of hydatid disease caused by *Echinococcus granulosus*: past, present and future. Int. J. Parasitol. 20, 431- 456.

Grosso, G., Gruttadauria, S., Biondi, A., Marventano, S., Mistretta, A., 2012. Worlwide epidemiology of liver hydatidosis including the Mediterranean area. World J. Gastroenterol. 18 (13), 1425-1437.

Hadrill, D., 2002. Horse healthcare: A manual for animal health workers and owners. ITDG Publishing. 256 pp.

371 Haridy, F.M., Abdel Gawad, A.G., Ibrahim, B.B., Hassan, A.A., El-Sherbi, G.T., El
372 Shazly, A.M., Morsy, T.A., 2008. Zoonotic hydatidosis in donkeys: post-mortem
373 examination in the zoo, Giza. Egypt. J. Egypt. Soc. Parasitol. 1, 305-312.
374
375 Lahmar, S., Kilani, M., Torgerson, P.R., Gemmell, M.A., 1999. *Echinococcus granulosus*
376 larvae in the livers of sheep in Tunisia: the effects of host age. Ann. Trop. Med.
377 Parasitol. 93, 75-81.
378
379 Lahmar, S., Debbek, H., Zhang, L.H., McManus, D.P., Souissi, A., Chelly, S., Torgerson,
380 P.R., 2004. Transmission dynamics of the *Echinococcus granulosus* sheep–dog
381 strain (G1 genotype) in camels in Tunisia. Vet. Parasitol. 121, 151–156.
382
383 Lahmar, S., Rebaï, W., Boufana, B.S., Craig, P.S., Ksantini, R., Daghfous, M.A., Chebbi, F.,
384 Ftériche, F., Bédioui, H., Jouini, M., Dhibi, M., Makni, M., Ayadi, M.S., Ammous, A.,
385 Kacem, M.J., Ben Safta, Z., 2009. Cystic echinococcosis in Tunisia: analysis of
386 hydatid cysts that have been surgically removed from patients. Ann. Trop. Med.
387 Parasitol. 103, 593–604.
388
389 Lahmar, S., Trifi, M., Ben Naceur, S., Bouchhima, T., Lahouar, N., Lamouchi, I., Maâmouri,
390 N., Selmi, R., Dhibi, M., Torgerson, P.R., 2013. Cystic echinococcosis in slaughtered
391 domestic ruminants from Tunisia. J. Helminthol. 87, 3, 318-325.
392
393 Majorowsky, M. M., Carabin, H., Kilani, M., Ben Salah, A., 2005. Echinococcosis in Tunisia:
394 a cost analysis. Trans. Royal Soc. Trop. Med. Hyg. 99, 268–278.
395
396 M'rad, S., Filisetti, D., Oudni, M., Mekki, M. Belguith, M., Nouri, A., Sayadi, T., Lahmar, S.,
397 Candolfi, E., Azaiez, R., Mezhoud, H., Babba, H., 2005. Molecular evidence of ovine
398 (G1) and camel (G6) strains of *Echinococcus granulosus* in Tunisia and putative role
399 of cattle in human contamination. Vet. Parasitol. 129, 267–272.
400
401 Mukbel, R.M., Torgerson, P.R., Abo-Shehada, M.N., 2000. Prevalence of hydatidosis
402 among donkeys in northern Jordan. Vet. Parasitol. 88, 35-42.
403

Nakao, M., Sako, Y., Yokoyama, Y., Fukunaga, M., Ito, A., 2000. Mitochondrial genetic code in cestodes. Mol. Biochem. Parasitol. 111, 415-24.

Roberts, M.G., Lawson, J.R., Gemmell, M.A., 1986. Population dynamics in echinococcosis and cysticercosis: Mathematical model of the life-cycle of *Echinococcus granulosus.* Parasitol. 92, 621–641.

Oge, S., Kircali, F., Yildirim, A., Oge, H., 2004. Hydatidosis (hydatid cyst) in equines. Ankara Univ. Vet. Fak. Derg. 51,75-76.

Schwartz, D., 1993. Méthodes Statistiques à l'Usage des Médecins et des Biologistes, 3rd Edn. Paris: Flammarion.

Smyth, J. D., Barrett, N.J., 1980. Procedures for testing the viability of human hydatid cysts following surgical removal, especially after chemotherapy. Trans. Royal Soc. Trop. Med. Hyg. 74, 649–652.

Thompson, R.C.A., McManus, D.P., 2001. Aetiology: parasites and life cycles. In: Eckert, J., Gemmell, M.A., Meslin, F.X., Pawlowski, F.X. (Eds), Manual on *Echinococcus* in Humans and Animals a Public Health Problem of Global Concern, WHO/OIE, Paris. World Health Organization, Geneva, pp. 1-19.

Torgerson, P.R., Williams, D.H., Abo-Shehada, M.N., 1998. Modeling the prevalence of *Echinococcus* and *Taenia* species in small ruminants of different ages in northern Jordan. Vet. Parasitol. 79, 35–51.

Torgerson, P.R., Shaikenov, B.S., Rysmukhambetova, A.T., Abdybekova, A.M., Usenbayev, A.E., Bairtursinov, K.K., 2003. Modeling the transmission dynamics of *Echinococcus granulosus* in cattle and sheep in Kazakhstan. Vet. Parasitol. 114, 143-153.

Utuk, A.E., Simsek, S., 2013. Molecular characterization of the horse isolate of *Echinococcus granulosus* in Turkey. J. Helminthol. 87, 3, 305-8.

437 Varcasia, A., Garippa, G., Pipia, A.P., Scala, A., Brianti, E., Giannetto, E., Battelli G,
438 Poglayen, G, Micagni, G., 2008. Cystic echinococcosis in equids in Italy. Parasitol.
439 Res. 102, 4, 815-8.
440
441

Tables and Figures.

Table 1. Species of *Echinococcus* from Tunisian donkeys according to host age, cyst location, fertility rates and protoscoleces viability.

Table 2. Organ location and lesional aspect of *Echinococcus* hydatid cysts from Tunisian donkeys

Table 3. Lesional aspect, fertility and protoscoleces viability of *Echinococcus* hydatid cysts from Tunisian donkeys according to age

Figure 1. Variation of the prevalence of CE in donkeys with age. Observed prevalence □ compared to model fit ■.

Figure 2. Variation of the abundance of *Echinococcus* cysts in donkeys with age. Observed abundance □ compared to model fit ■.

Table 1
Species of *Echinococcus* from Tunisian donkeys according to host age, cyst location, fertility rates and protoscoleces viability.

N° infected donkey	Age (years)	Sex	Age-Group (years)	Total fertile cysts	Total typed fertile cysts	Organ location	Strain	Mean fertility (protoscoleces/ml)	Mean viability
64	2	Male	≤ 2	2	1	Lung	*E equinus*	7,281	80%
955	7	Female	3 to 9	12	7	Liver	*E equinus*	8,812	67.10%
978	4	Male			2	Liver	*E equinus*	N/D	N/D
158	15	Female	10 to 19	16	1	Lung	*E granulosus*	15,112	65.78%
238	12	Female			1	Liver	*E granulosus*	N/D	N/D
346	19	Female			7	Lung	*E granulosus*	N/D	N/D
695	15	Female			1	Lung	*E granulosus*	N/D	N/D
775	15	Male			3	Lung	*E granulosus*	N/D	N/D
800	20	Female	≥ 20	13	10	Liver	*E equinus*	7,246	67.16%
103	20	Male			2	Liver	*E equinus*	N/D	N/D

N/D, Not done

Table 2
Organ location and lesional aspect of *Echinococcus* hydatid cysts from Tunisian donkeys

Cyst location	Number and (%) of donkey cysts											
	Observed		Viable		Fertile		Caseous		Calcified		Fertility	Viability
	N	%	N	%	N	%	N	%	N	%	Protoscoleces/ml	(%)
Liver	810	89.9	290	35.80	29	3.58	0	0	521	64.32	10,229	81.50
Lungs	91	10.09	30	32.96	14	15.38	9	9.89	51	56.04	2,961	76.59
All	901	100	320	35.51	43	4.77	9	0.99	572	63.48	13,190	79.04

N, number; ($\chi 2 = 96.50$; $p = 0.000000$)

Table 3
Lesional aspect, fertility and protoscoleces viability of *Echinococcus* hydatid cysts from Tunisian donkeys according to age

	Number and (%) of donkey cysts										
Age (Years)	Observed cysts	Viable N	Viable %	Fertile N	Fertile %	Caseous N	Caseous %	Calcified N	Calcified %	Fertility Protosc./ml	Viability (%)
≤ 2	46	4	8.69	2	4.34	0		42	91.30	14,562	90.28
3 to 9	133	50	37.59	12	9.02	4	3	79	59.39	11,750	70.10
10 to 19	242	100	41.32	16	6.61	5	2.06	137	56.61	18,600	84.80
≥ 20	480	166	34.58	13	2.70	0		314	65.41	7,850	71
All	901	320	35.51	43	4.77	9	0.99	572	63.48	13,190	79.04

Figure

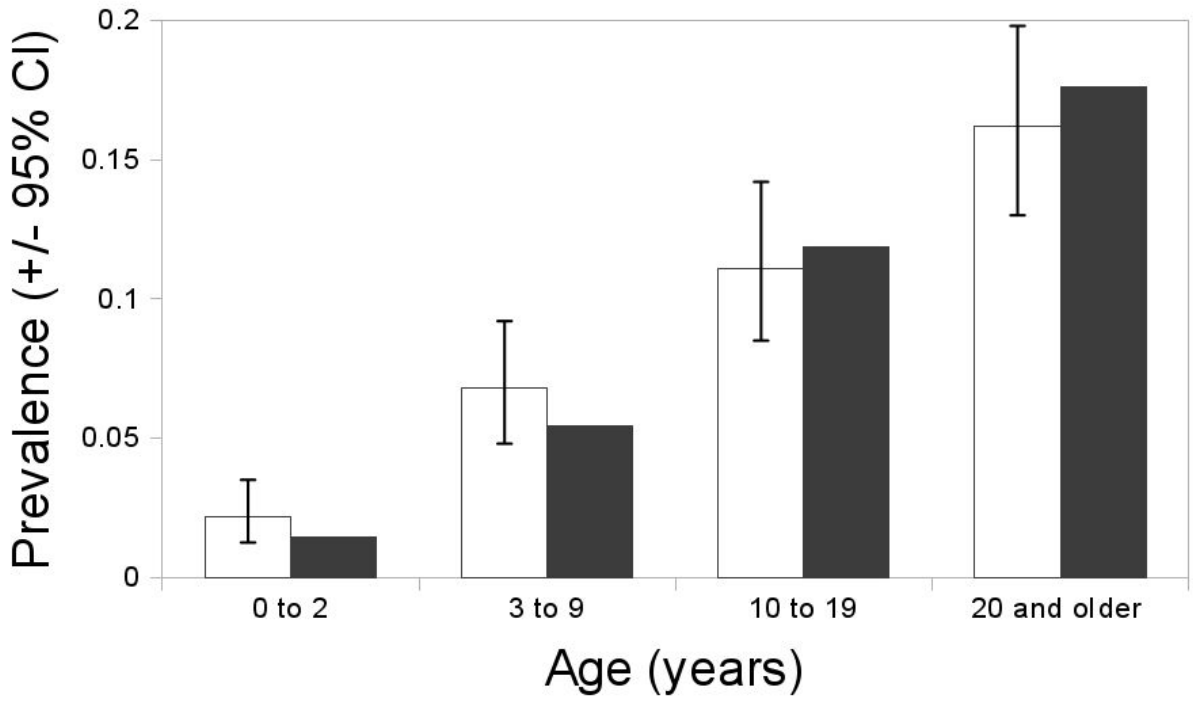
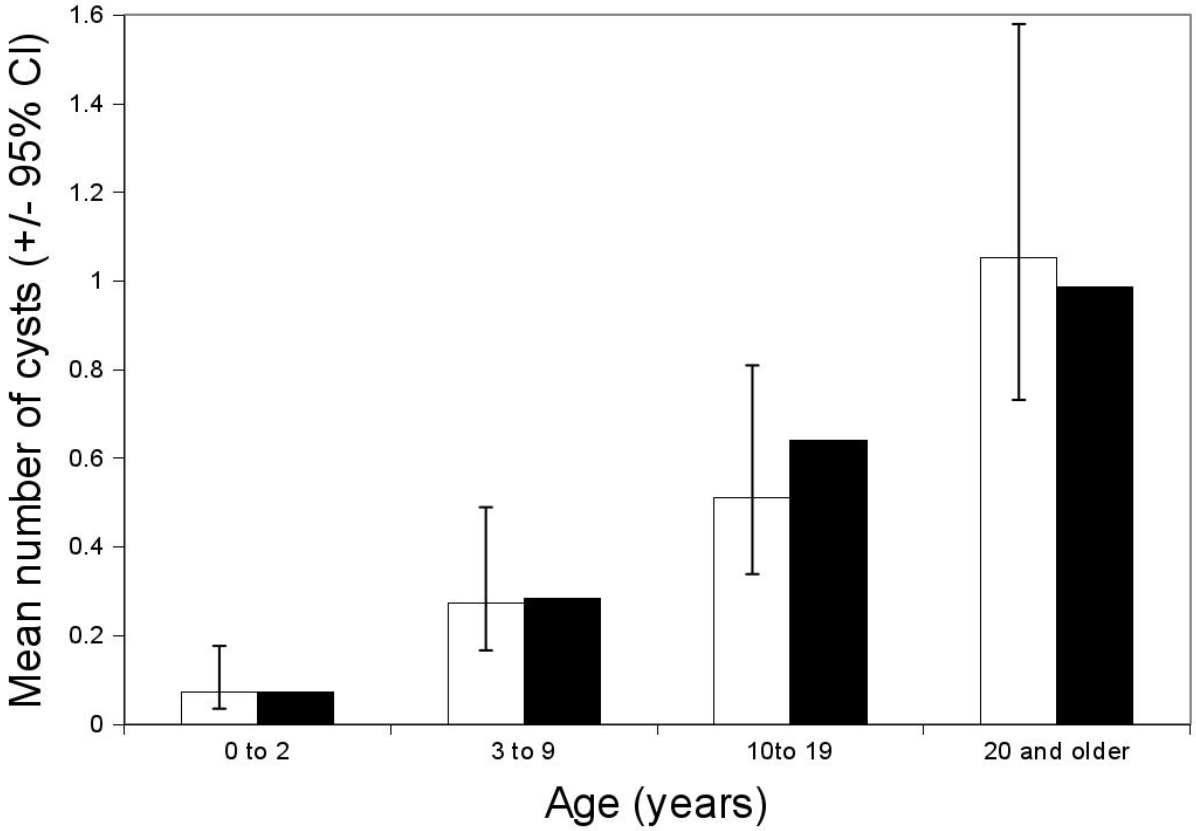


Fig. 1. Variation of the prevalence of CE in donkeys with age. Observed prevalence □ compared to model fit ■.

Figure

1



2 **Fig. 2.** Variation of the abundance of *Echinococcus* cysts in donkeys with age. Observed abundance □
3 compared to model fit ■.

4

5